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Research report

The response of human and rat fetal ventral mesencephalon in culture to the brain-derived neurotrophic factor treatment

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Abstract

Brain-derived neurotrophic factor (BDNF) has been shown to increase the survival of dopaminergic neurons in rodent mesencephalic cultures. The mRNAs of BDNF and *trk B* receptor have been found to be expressed in the substantia nigra of rat. In this study, the action of BDNF was studied on the survival and transmitter-specific differentiation of dopaminergic neurons of fetal human CNS aged 9–10-week in vitro. Dopaminergic neuron viability and phenotypic expression were monitored by tyrosine hydroxylase (TH) immunohistochemistry and measurement of dopamine (DA) content with HPLC, respectively. After seven days of treatment with BDNF there were 2.2-fold greater number of TH⁺ neurons surviving than in untreated cultures. Although very low levels of DA were detectable in human tissue, considerable amounts of DA was found in the culture medium from around 13 days in vitro (DIV), indicating that DA in human fetal tissue tended to be synthesised and released into the incubation medium more readily than from cultured rat fetal tissue during the same period. The content of DA in the BDNF-treated cultures was approximately double that of untreated cultures after 7 days. In rat fetal tissue, the capacity of each TH⁺ neuron to produce DA was not changed in the BDNF-treated cultures (7 DIV) compared with control cultures, suggesting that BDNF does not up-regulate the production of DA but rather acts to reduce cell death rates. Ciliary neurotrophic factor (CNTF) treatment of rat mesencephalic culture failed to improve the period of survival of fetal dopaminergic neurons and had no effect on the production of DA in cultures. Taken together, our results suggest that BDNF has potent trophic effect on both rat and human fetal mesencephalic dopaminergic neurons in culture and has a potential application in the treatment of Parkinson's disease.

Key words: Brain-derived neurotrophic factor; Cell culture; Dopaminergic; Survival; Rat; Human

1. Introduction

Parkinson's disease is a neurodegenerative condition, the most prominent pathological feature of which is progressive loss of mesencephalic dopaminergic neurons. Striatal dopamine originates in these cells, loss of which is considered to underlie the appearance of motor disability. A number of strategies have been developed to restore the level of striatal dopamine in patients with Parkinson's disease. One of the most promising approaches is the transplantation of embryonic mesencephalic dopaminergic neurons into the striatum. There is now much evidence that certain

growth/trophic factors are able to increase the relatively poor survival of neurons in vivo and maintain declining neurons during in vitro culture [20,21,29,30]. Treatment with neurotrophic factors is one of the options to improve their decreased viability both in vitro and in vivo.

A number of trophic substances have been shown to enhance the survival of mesencephalic dopaminergic and other neurons. Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family, which includes nerve growth factor (NGF), neurotrophin-3 (NT-3), and NT-4/5. BDNF is a growth factor known to act relatively specifically on dopaminergic neurons. Others, such as insulin, insulin-like growth factors (IGF-1, IGF-2) [4,20], acidic/basic fibroblast growth factor (aFGF, bFGF) [4,8,15,20] and epidermal growth factor (EGF) [5,20], all influence dopaminergic neu-

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rons non-specifically [4] as well as acting on non-neuronal cell, such as astroglial cells [3,8,16,23,35,36]. Previous studies have shown that *trk* B and low-affinity NGF receptors (p75-NGFR), form the BDNF receptors, and mediate its neurotrophic effects [6]. CNTF was originally described as a neurotrophic factor acting on cholinergic neurons of the chick ciliary ganglion [1]. It has been reported that exogenous CNTF prevents the degeneration of axotomized neurons *in vivo* [13,39]. These data support the perception that CNTF is an injury factor or lesion factor. There is evidence that CNTF receptors are widely distributed in many regions of the central nervous system (CNS), including neocortex, and substantia nigra pars reticulata etc, except for peripheral nervous system (PNS) as expected. The expression of CNTF receptor is found exclusively in neurons [19]. There have been no published reports of the effect of BDNF on human mesencephalic neurons and information on the potential ability of CNTF to influence dopaminergic neurons *in vitro* is lacking. In the present study, we investigated the potential trophic effect of BDNF on both human and rat fetal dopaminergic neurons *in vitro* and action of CNTF on rat dopaminergic neurons in culture. Our results suggest that BDNF, but not CNTF, has a potent trophic effect on fetal rat dopaminergic neurons *in vitro*. BDNF also has a similar potent action on human dopaminergic neurons in culture.

2. Materials and methods

2.1. Cell preparation

Human fetuses (8-10-week-old) were obtained from either curettage abortions or from drug RU486-induced abortion after fully informed consent. Material was taken with the consent of the patients and the agreement of the local Ethical committee. The age of the embryos was established by morphological assessment according to multiple criteria [32] e.g. the length of the fetuses if they were relatively intact, the distinguishing developmental characteristics on the fetuses and timing of the patients menstrual periods. Rat fetuses were obtained from pregnant Sprague-Dawley rats at day 14-15 (E14.5. E0 = day of vaginal plug) of gestation. Fetal cadavers and rat embryos were stored in ice-cold Hank's balanced saline solution (Ca^{2+} and Mg^{2+} free, HBSS) until dissection. The mesencephalon and myelencephalon were dissected out and placed in HBSS. After removal of meninges, the tissue was cut into 0.2 cm sections. Following washing the tissue was then incubated in glucose saline containing 1 mg/ml trypsin and 0.5 mg/ml DNase at 37°C for 15 min. Tissue was then sequentially washed in glucose-saline containing DNase.

2.2. Cell cultures

Either fetal human cells or rat cells were seeded into 35 mm plastic Petri dishes (Falcon) or 16 mm multiwell plates (Nunc) precoated with poly-L-lysine (5 $\mu\text{g}/\text{ml}$) as described by Walters et al. [44]. Cell viability in suspensions of dissociated cells was determined by the ability of viable neurons to exclude the dye Trypan blue. The

cells were plated at $2-4 \times 10^6$ cells per Petri dish ($2-4 \times 10^5$ cells/ cm^2) or 5×10^5 cells per multiwell (2.5×10^4 cells/ cm^2) containing 2 ml or 0.5 ml of Dulbecco's modified Eagle's medium (DMEM) or RPMI 1640 supplemented with 10% fetal calf serum (FCS), 2 mM L-glutamine, 25 mM KCl and 50 $\mu\text{g}/\text{ml}$ gentamycin, and incubated at 37°C in a 95% air/5% CO_2 humidified atmosphere. For the study of trophic factor action, a cell density of $2-2.5 \times 10^5$ cells/ cm^2 was used. The medium was changed approximately every 5 days. The cells were taken for biochemical determination of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), and for tyrosine hydroxylase (TH) immunocytochemistry at different time points.

2.3. Experimental treatment

Recombinant human (rh) BDNF and ciliary neurotrophic factor (CNTF) were provided by Regeneron Pharmaceutical Ltd., USA. These growth factors were prepared in sterilised 0.1 M phosphate buffered saline (PBS) containing 5% FCS. BDNF (50 ng/ml) was added to human and rat mesencephalic cultures every other day, starting from the day of plating out the culture (= day 0) until 7th day *in vitro* (DIV) in rat and 23 DIV in human. CNTF (50 ng/ml) was applied in rat mesencephalic cultures in the manner as above until 7th DIV.

2.4. Measurements of DA and DOPAC by high pressure liquid chromatography (HPLC)

2.4.1. Tissue

The presence of DA and DOPAC was measured in the cultures by HPLC coupled to electrochemical detection according to the method of Walters et al. [44] with a few modifications. In brief, these cultures were rinsed with PBS prior to harvesting in 500 μl of 0.1 M PBS. Protein in the samples was precipitated with 0.4 M HCl, and the tissue was homogenised at 4°C. The extracts were then centrifuged $10,000 \times g$ for 30 min at 4°C. The supernatants were dried down by vacuum concentrator. The pellets were placed in 100 μl of 0.04 M perchloric acid. After centrifugation, the supernatants were taken for analysis.

2.4.2. Culture medium

To detect DA and DOPAC in the culture medium, samples of medium were taken when each routine medium change was made. This medium contained ascorbic acid (50 mg/l) and was changed approximately every 5 days. The extraction method was exactly same as that for tissue. The chromatographic system comprised a reverse-phase octadecylsilane column (ESA HR80 3 mm column, dimensions 8 cm \times 4.6 mm); the mobile phase was ESA Cat-A Phase reagent with a flow rate of 1.5 ml/min. An ESA 5011 analytical cell was employed with applied potentials of 0.00 V at detector 1 and +0.35 V at detector 2 coupled to an ESA Coulochem 5100A controller. An ESA 5020 guard cell with an applied potential of +0.40 V was positioned prior to the auto-sampler. Samples of the cell/medium extracts (20 μl) were analysed. The results were expressed as pmol catecholamine per mg protein, or pmol per ml of medium. The internal standard used was 3,4-dihydroxybenzylalanine (DHBA). The detection limit of this system is 10 fmol for DA, 20 fmol for DOPAC.

2.5. TH immunocytochemistry

Cultures were washed with Earle's balanced salt solution (EBSS), fixed with 4% paraformaldehyde for 30 min, and preincubated in EBSS containing 10% goat serum. They were then incubated for 48-72 h with a rabbit anti-TH serum (a kind gift from Dr. Tong Joh, Cornell University Medical College, New York, USA) diluted 1:8,000 in EBSS containing 0.1% Triton X-100. After washing, the cells were

incubated for 45 min with a biotinylated goat anti-rabbit IgG fraction (Vector, USA) diluted 1:200 in EBSS. They were washed again with EBSS and incubated for 45 min with a preformed avidin-biotinylated horseradish peroxidase complex diluted 1:100 in EBSS. The peroxidase was revealed by incubation with a solution of 0.5 mg/ml diaminobenzidine in EBSS containing 0.015% hydrogen peroxide. Following staining, coverslips were cleared and mounted using DPX mountant. The number of cells in a culture dish was counted by microscopic analysis. The number of TH⁺ neurons in rat cell cultures were counted in eight randomly chosen observation fields per well or Petri dish. The number of TH⁺ cells in human mesencephalic cultures were counted by screening entire cultures.

2.6. Protein measurement

Human cultures were washed with PBS and extracted as described for the DA assay. The pellets, after centrifugation, were used for protein assay according to the method of Lowry [24], using bovine serum albumin as a standard.

2.7. Statistical evaluation

Some results from the DA assay and counting of TH⁺ cells are expressed as a percentage of the control values to stabilize the variance between individual experiments. The data were submitted to either a Student *t*-test or a one-way analysis of variance (ANOVA) followed by the Student–Newman–Keul's *t*-test as a post-hoc test for difference between the groups. Difference were considered statistically significant at *P* < 0.05.

3. Results

3.1. Development of fetal human and rat dopaminergic neurons in culture

3.1.1. Rat cultures

Rat ventral mesencephalon was taken from 14-day-old rat pups. At this point the dopaminergic neuron-

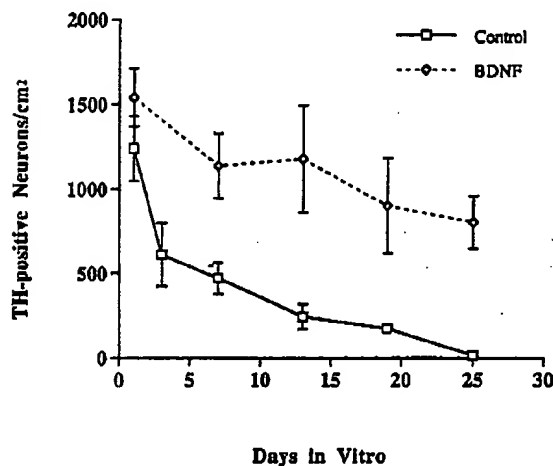


Fig. 1. Time-course of neuronal survival in control and BDNF-treated cultures. Rat mesencephalic cells were grown for various days in RPMI 1640 with 10% fetal calf serum (FCS), 2 mM L-glutamine and 25 mM KCl. Recombinant human (rh) BDNF (50 ng/ml) was added to the culture medium every other day starting immediately after plating. Bars indicate mean \pm S.E.M. (*n* = 6).

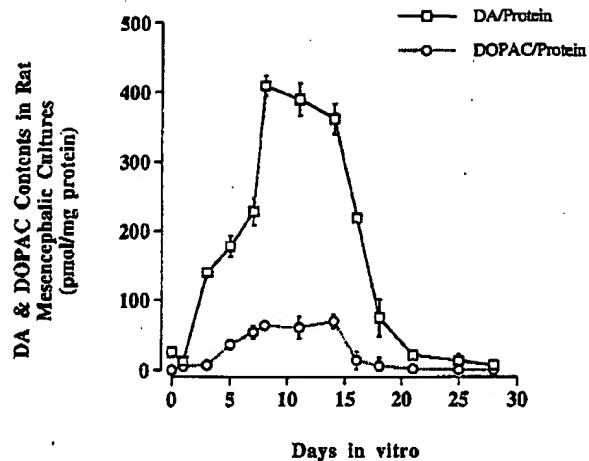


Fig. 2. Time-course of production of DA and DOPAC in control rat mesencephalic cultures. Rat mesencephalic cells (E14) were plated at 4×10^6 cells per 35 mm Petri dish and cultures were taken for measurement of DA and DOPAC with HPLC at different time points. Graph shows means \pm S.E.M. (*n* = 5–6).

containing tissue usually yielded cells with a higher viability and has commonly been used at this age in this laboratory [44] and others for neural transplantation into animal model of Parkinson's disease. Rat cells usually attached to the plates within 12 h of plating. At the time of plating, most of the neurons had short

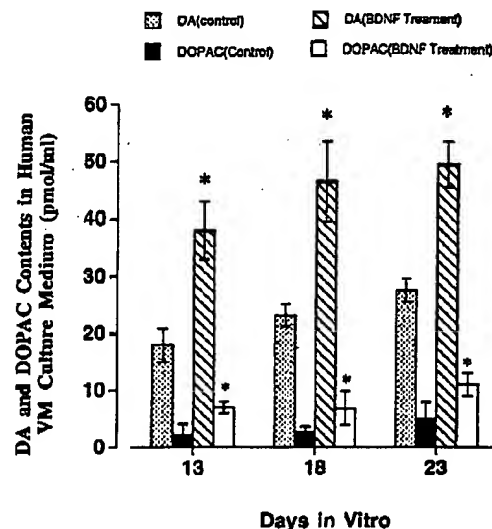


Fig. 3. Effects of rhBDNF on human fetal mesencephalic neurons. Human mesencephalic cultures were grown in DMEM medium with 10% FCS, 2 mM L-glutamine and 25 mM KCl. rhBDNF (50 ng/ml) was added every other day after plating. Culture medium was collected every 5 days and DA was measured in the culture medium with HPLC. Bars indicate means \pm S.E.M. (*n* = 4). Asterisks indicate statistical difference from control group at individual time points (*P* < 0.01). Note: Almost all of the DA was recovered in the culture medium from human fetal cells rather than that in the tissue itself.

processes. The majority substantially extended their neurites during 24 h after culture. However, the number of TH⁺ neurons was declining continuously after plating (Fig. 1). More than 50% of TH⁺ neurons were lost within 3 DIV, suggesting that the first couple of days after plating is a crucial time period for maintaining these dopaminergic neurons. By 13 DIV, there were only some 20% of TH⁺ neurons left in the cultures. Many of TH⁺ neurons developed neurites.

Some of these cultures were extracted for measurement of DA and DOPAC. There was very little DA and DOPAC detected in cultures immediately after plating (day 0) and in 1-day-old mesencephalic cultures. With time, the level of DA and DOPAC (Fig. 2) in the tissue rose gradually to reach a maximum around 8-14 DIV.

Cell density effects on survival of TH⁺ neurons was investigated. Survival was maximal at a seeding density

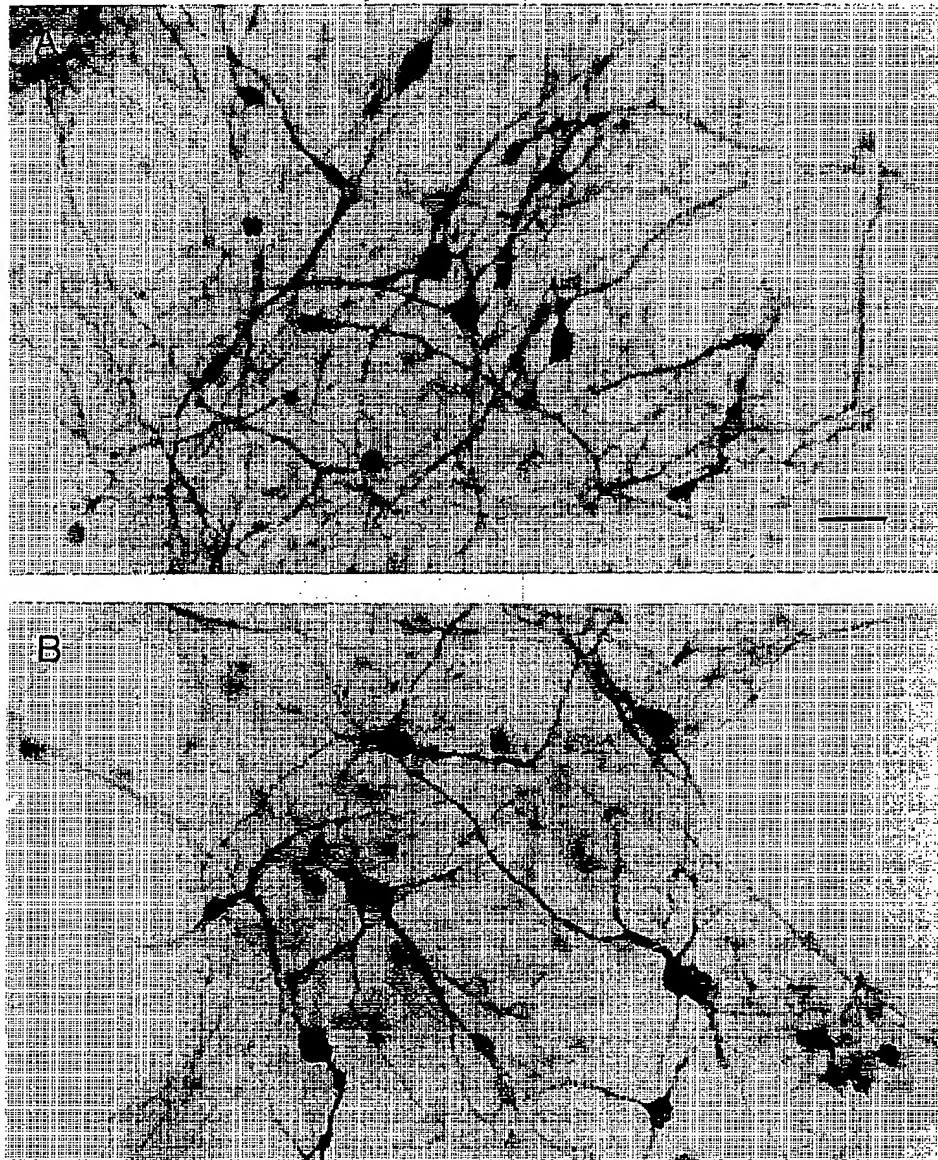


Fig. 4. Photomicrograph of cultured human fetal mesencephalic cells from 8-week-old fetus (A, C) and fetal rat mesencephalon (E14) (B, D) immunostained for TH as described in Methods section. Human and rat mesencephalic cells were grown for 23 and 7 days in growth medium, respectively. A, B: BDNF treated. C, D: Controls (no addition). Bar = 50 μ m.

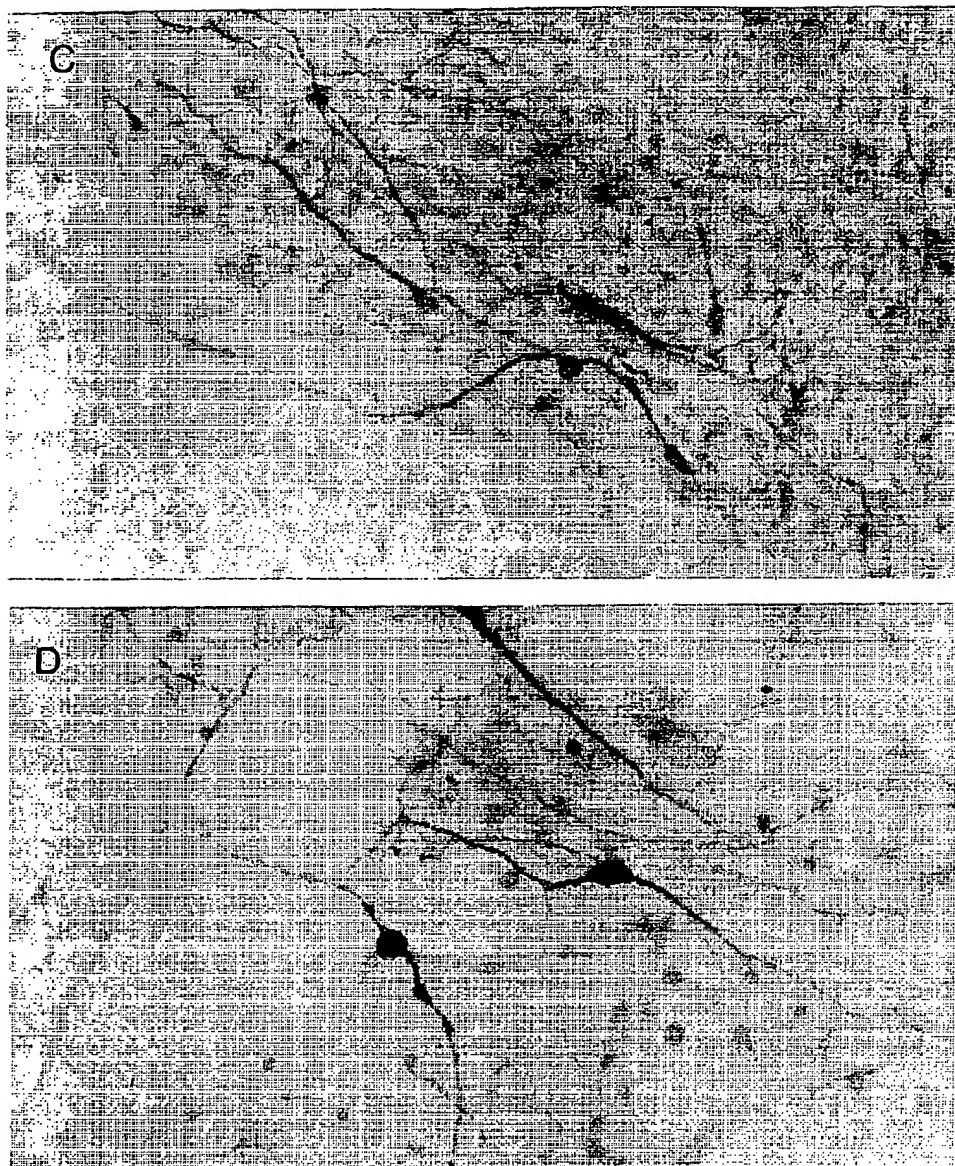


Fig. 4 (continued).

of $2 \times 10^5/\text{cm}^2$ and decreased at seeding density of $0.7\text{--}1 \times 10^5/\text{cm}^2$. No TH⁺ cells were visible in the cultures 3 DIV at a density lower than $5.4 \times 10^4/\text{cm}^2$.

It was previously reported from this research group [44] that rat fetal mesencephalic cultures contain between 0.1 ~ 1.0% of TH⁺ neurons.

3.1.2. Human cultures

Human fetal mesencephalic cells took 24–36 h to attach to the Petri dishes which is longer than that for rat cells (i.e. 12 h). At the same time they started to extend their neurites. Human fetal dopaminergic neu-

rons could consistently be maintained in vitro for more than 7 weeks, whereas rat dopaminergic neurons lived for only 3–4 weeks, indicating that under the culture conditions used here human cells were viable for a longer period of time than the rat cells. The level of DA from cultured human mesencephalon from day 1 to 21 after plating was monitored. Only small amounts of DA were detectable in the tissue itself, (Table 1) even though the human midbrain area dissected out from the fetuses was rich in TH-positive neurons, and also a substantial number of TH-positive neurons were visualised in these cultures (Fig. 4A,C).

Table 1
Dopamine content in human mesencephalic cultures

Days in vitro	1	3	9	17	21
Dopamine (pmol/mg protein)	0	0	2.3 ± 0.2	3.4 ± 0.4	3.08 ± 0.2

Human mesencephalic cultures were grown in DMEM medium with 10% FCS, 2 mM L-glutamine and 25 mM KCl. DA was measured in the tissue itself with HPLC. Means ± S.E.M. ($n = 4$). Gestational ages of human fetuses were 8–10 weeks.

A previous report from this group showed that between 0.1 and 0.5% of neurons present in these human mesencephalic cultures were TH-positive [44].

Considerable amounts of DA and DOPAC were found in the culture medium from around 13 DIV (Fig. 3), indicating that DA in human fetal tissue tended to be synthesised and released into the incubation medium more readily than from cultured rat fetal tissue during the same period. At 8 DIV only a very low level of DA was detectable (see also Table 1).

3.2. The trophic actions of BDNF

3.2.1. Neurotrophic effect of BDNF on survival of human fetal dopaminergic neurons

Neuronal survival. Previous cell culture studies of rat tissue showed that BDNF can provide trophic support for different types of neurons from several brain regions including hippocampus and mesencephalon. It was therefore of interest to determine whether BDNF was also trophic for human central dopaminergic neurons. Cultures of human mesencephalon were maintained in the absence or presence of 50 ng/ml of BDNF and survival of TH-positive neurons was determined by counting the neurons in the cultures on day 23. In untreated cultures there was a progressive loss of TH-positive neurons over the course of the experiment. By culture day 13 greater than 50% of dopaminergic neurons has died (Table 2). Survival of these neurons was significantly enhanced in the presence of BDNF (Fig. 4). After longer periods in culture (e.g. 33, 38, 113 DIV) TH-positive neurons were still visible but

Table 2
The effect of BDNF on TH-positive neurons in human mesencephalic cultures

Days in vitro	1	13	23
Control (cells/cm ²)	755 ± 11	362 ± 13	218 ± 28
BDNF (cells/cm ²)	732 ± 10	676 ± 19 ^a	589 ± 34 ^b

Cultures prepared like those of Table 1 were fixed at different time points as above and taken for immunocytochemical visualization of TH. The number of TH-positive neurons was determined in eight randomly chosen observation fields per Petri dish and expressed as cells/cm². Mean ± S.E.M.; $n = 3$. Gestational ages of human fetuses were 9–10 weeks.

^a Higher than control value at 13 DIV. $P < 0.05$.

^b Higher than control value at 23 DIV. $P < 0.05$.

much reduced in number (<5%) in the absence of added BDNF. Also BDNF treatment induced the dopaminergic neurons to extend more neurites per cell body and induced more branching of neurites as compared to controls as judged by visual inspection of the cultures (Fig. 4A,C).

DA synthesis. As reported above, the DA produced by human fetal tissue, was recovered mainly in the incubation medium (Fig. 3). The synthesis of the compound was approximately doubled by addition of BDNF (50 ng/ml). This enhancement was seen up to at least 23 DIV. Dopamine was produced by the BDNF-treated human cultures up to at least 38 DIV.

3.2.2. Neurotrophic effect of BDNF on survival and differentiation of rat fetal dopaminergic neurons

Time-course of neuronal survival in control and BDNF-treated rat fetal cultures. The typical survival pattern of TH-positive neurons is shown in Fig. 1. Whereas the numbers of TH-positive neurons declined virtually to zero after 25 days in control cultures, they were well maintained in cultures treated with BDNF. All effects were significant ($P < 0.05$).

DA synthesis. As in human mesencephalic cultures, BDNF at 50 ng/ml applied every other day doubled DA levels in rat tissue. The DA quantitated in control cultures was 79.5 ± 1.7 pmol/dish and 191.5 ± 2.9 pmol/dish in the BDNF treated cultures. The effect was significant ($P < 0.05$).

Other trophic effects. The effect of BDNF on the functional differentiation of dopaminergic neurons was determined by estimating the number of TH-positive neurons in half of the cultures and measuring the DA content of tissue in the sister cultures. The DA produced in control cultures (7 DIV) was 2.11 ± 0.1 (mean ± S.E.M., $n = 3$) fmol/neuron and 1.89 ± 0.08 (mean ± S.E.M., $n = 3$) fmol/neuron in the BDNF-treated cultures. The difference between these two groups was not significant.

3.3. Action of CNTF

CNTF at 50 ng/ml did not influence the production of DA in rat fetal cultures during alternative day treatment for 7 days. At day 7 the DA content was $85.6 \pm 2.8\%$ ($n = 3$) of control in CNTF-treated mesencephalic cultures. There was no significant difference between these two groups.

4. Discussion

4.1. Promotion of neuronal survival by BDNF

Our results demonstrate that BDNF has a powerful positive effect on the survival of human and rat mesen-

cephalic dopaminergic neurons in culture. Previous studies have shown that basic fibroblast growth factor (bFGF) has mitogenic action on primary neurons from hippocampus [37] and substantia nigra [28]. However, we are confident that the trophic effect of BDNF on dopaminergic neurons of the age used in this study is due to promotion of survival rather than cell proliferation. In a scanning electron microscope study (data not shown) dividing dopaminergic neurons were never found in rat mesencephalic culture treated with BDNF (50 ng/ml). Also, BDNF (100 ng/ml) was shown to be unable to stimulate the expression of TH gene [4]. Therefore, the 50 ng/ml dose level of BDNF used in this study is unlikely to have exerted its effective stimulation of the proliferation of dopaminergic cells. Others [7,17] have reported that BDNF-like, and pure BDNF, respectively, promote the survival of dopaminergic neurons in culture. Delayed addition of BDNF which provided evidence against its having an action by enhancing TH gene expression [17].

Our results showed the importance of cell density of mesencephalic cells on their survival in culture. This is consistent with the finding of Engele et al. [9], indicating that cellular interaction is involved in their survival. However, cellular interaction between mesencephalic cells has not been widely reported. Thus rat dopaminergic neurons are shown to be responsive to BDNF in our studies and those of others [17,21]. Also mRNAs for BDNF and for *trkB* receptors have been detected by in situ hybridization and northern blot analysis, respectively, in substantia nigra neurons from both adult brain and developing ventral mesencephalon in cultures [12,18,31]. This strongly suggests that BDNF is very likely functioning as an autocrine growth factor, or as an intracellular regulatory substance in rat CNS. It remains to be investigated whether developing human mesencephalic cells have the capacity to synthesize BDNF. There is an evidence that *trkB* and BDNF mRNA are expressed in human neuroblastomas [33]. The trophic effect of BDNF on human dopaminergic neurons in the present study indicates that *trkB*, the functional high-affinity receptor for BDNF, exists in cultures of developing human as well as rat mesencephalic cells. Furthermore, the receptor-mediated signal transduction evoked by BDNF in the two species may be identical.

Previous in vitro studies have shown that factors derived from the striatum can influence the differentiation of central dopaminergic neurons [7,41]. Also studies of the development of rat mesencephalon have demonstrated that dopaminergic cell bodies appear at developmental stages E12–15 and the target area of innervation develops gradually from E13 to postnatal periods [40,43]. This raises the question of whether striatum synthesizes BDNF which influences the embryonic development of mesencephalic cells. Indeed

there is evidence that the striatum contains only very low levels of BDNF mRNA during development and in the adult [11,27]. Therefore, during the early developmental stages, (e.g. E12–15) the striatum may not contain sufficient BDNF to influence the differentiation of dopaminergic cells. However, locally produced trophic factors including BDNF could be available from this source to influence the development of nigrostriatal neurons.

4.2. Specificity of action of BDNF

Previous studies have shown that BDNF is able to up-regulate ChAT expression and GABA uptake in cholinergic and GABAergic neurons, respectively [2,21], indicating that BDNF can act on a range of functional properties of a variety of neuron types. It is not yet clear whether the presence of non-dopaminergic neurons (or even glial cells) is necessary for BDNF to show its action on dopaminergic neurons.

4.3. Dopamine production

In rat mesencephalic cultures treated with BDNF, the production of DA was approximately doubled. However, when the TH-positive neurons present were counted, it became clear that the DA production per neuron remained constant. This finding further supports the conclusion that BDNF acts primarily to promote dopaminergic neuron survival rather than up-regulating TH gene expression or promoting dopaminergic cell proliferation.

Previous studies have shown the earliest date of appearance of TH⁺ neurons in fetal human mesencephalon is 5–6.5-weeks of embryonic age [10,42]. The monoamine and metabolite levels in fresh and cryopreserved human fetal mesencephalic tissue of 7–10 weeks fetal age have previously been reported [22]. The results reported here provide the first evidence of biochemical performance of human fetal mesencephalic dopaminergic neurons in the presence or absence of BDNF in culture. The ratio of DA/DOPAC in our both control and BDNF-treated cultures at 13 DIV is about 9:1, which is consistent with the published findings of other authors [22]. These same authors also showed that substantial amounts of DA were present in 7–10-week-old fetal human mesencephalon [22]. However, DA was not detected in human mesencephalic cultures in the early stages after plating in the present study, although the dissected mesencephalic tissue was from an area enriched in TH⁺ neurons, and there were substantial numbers of TH⁺ cells identified in the cultures employed in the study. A possible reason for this difference is that the number of dopaminergic neurons used here for measurement was rather small compared with cell numbers used by these

previous authors [22]. Therefore the DA content of the cells in each culture dish could have been too low to be detectable by the method employed here. About $8 \times 10^6 \pm 1.9 \times 10^6$ ($n=8$) cells were obtained from each tissue sample, and plated as 2×10^6 cells per Petri dish, i.e. approximately one-fourth of original mesencephalon tissue in one dish. The whole of each dish was taken for DA/DOPAC measurement. Moreover, there was significant loss (at least 50%) of dopaminergic cells in culture due to cell death during the first week after plating. Therefore the DA content in each culture dish was likely to have been too low to be detectable as this represented about one quarter to one third of the number of dopaminergic cells taken for DA measurement by these authors [22].

The finding that almost all of the DA was recovered in the culture medium from human fetal cells rather than that in the tissue itself was unexpected. This could be due to a greater sensitivity of these neurons to culture conditions, or indicate that the neurotransmitter storage system (e.g. synaptic vesicle), or regulation mechanism for neurotransmitter release in these fetal neurons, is not developed as well as in rat tissue of equivalent age. It is to be noted that 25 mM KCl was employed in the culture medium, and is theoretically at a membrane-depolarising concentration. However, the fact that the DA and DOPAC present in rat tissue cultures, was recovered mainly in the tissue rather than the culture medium, indicates that depolarization-induced release from cultured human tissue is unlikely to explain DA and DOPAC recovery in the medium.

4.4. Action of CNTF on rat fetal DA cells

CNTF has been shown to have several biological actions in the CNS and PNS. CNTF promotes the survival of spinal motoneurons [25,34] and protects adult septal cholinergic and non-cholinergic neurons against axotomy-induced damage [13]. Whilst there is increasing evidence for a widespread role of CNTF on CNS neurons, its action on central dopaminergic neurons has not been fully investigated. CNTF was shown by others to induce choline acetyltransferase (ChAT) and decrease TH activity in cultured rat sympathetic neurons [38], suggesting that CNTF does not maintain TH expression in neurons and tends to influence immature neurons towards the cholinergic phenotype. The finding [14] that the survival of dopaminergic neurons in substantial nigra was promoted by CNTF treatment following transection of the rat nigrostriatal pathway, but lost their TH immunoreactivity, further supports the speculation that CNTF down-regulates TH expression in central dopaminergic neurons. In the present study CNTF, unlike BDNF, was not able to reduce the rate of dopaminergic cell death or increase the production of DA in rat mesencephalic culture. A

decreased TH enzyme activity could be one of the reasons. Others have reported that concurrent treatment with 5 μ M DA and CNTF enhanced the expression of TH immunoreactivity in cultured rat mesencephalic neurons, although this treatment was not able to support the survival of TH-positive neurons [26]. In our rat dopaminergic cultures, the level of DA accumulating in the incubation medium following release from dopaminergic neurons, was far less than 5 μ M.

In conclusion, the present study examines the effect of BDNF on both human and rat embryonic dopaminergic mesencephalic neurons and the action of CNTF on rat cultures. BDNF has potentially promoted the survival of cultured human/rat fetal dopaminergic cells. CNTF has no significant action on rat fetal dopaminergic neurons in cultures. These findings add impetus to further exploration of the therapeutic potential of BDNF in the treatment of Parkinson's disease.

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